

SEQUENCE LISTING

<110> Chauhan, Sarita  
DiCosimo, Robert  
Payne, Mark  
Gavagan, John  
Fallon, Robert

<120> Isolation and Expression of a Gene for Nitrilase from  
Acidovorax Facilis 72W

<130> BC-1032 US NA

<140>  
<141>

<150> 60/193,707  
<151> March 31, 2000

<160> 32

<170> Microsoft Office 97

<210> 1  
<211> 17  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Forward primer  
(1F)

<220>  
<223> K= G or T, M= A or C, S= G or C, Y= C or T

<400> 1  
tkkmtkccsg gctaycc  
17

<210> 2  
<211> 17  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Reverse primer  
(7R)

<220>  
<223> S= G or C, H= A or C or T, M= A or C, R= A or G,  
Y= C or T

<400> 2  
ggccasshtg mrayrtg  
17

<210> 3  
<211> 385  
<212> DNA  
<213> Acidovorax facilis

<400> 3  
 ctattgggcg tggctcgcg acgtgaagta cagcctaagc ttacttcac gctatcacga  
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 gaattcgttg gagctagtg acgaccgat gcgtcgctc cagctggccg cgcgcgcaa  
 120  
 caaaatcgca ctctcatgg gctattcgga gcgggaagcc ggatcgctc atctgagcca  
 180  
 ggtgttcatc gacgagctg gcgagatctg tgccaatcgg cgcaagctga agcccacaca  
 240  
 cgttgagcgt acgatctacg gcgaaggcaa cggaaccgat ttctcaccg agacttcgc  
 300  
 gttcggacgc gtcggtggat tgaactgctg ggaacatttc caaccgtca gcaagttcat  
 360  
 gatgtacagc ctcggtgagc aggtc  
 385

<210> 4  
 <211> 1110  
 <212> DNA  
 <213> *Acidovorax facilis*

<400> 4  
 gtgggttcgt ataacagcaa gttctcgcg gcaaccgttc aggcagagcc ggtatggctc  
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 gacgcagacg caacgatcga caagtcgatc ggcatcatcg aagaagctgc caaaagggc  
 120  
 gcgagcttga tcgctttccc ggaagtattc attccgggct accctattg ggcgtggctc  
 180  
 ggcgacgtga agtacagcct aagctttact tcacgctatc acgagaattc gttggagcta  
 240  
 ggtgacgacc gtatcgctcg cctccagctg gccgcgcgc gcaacaaaat cgactcgtc  
 300  
 atgggctatt cggagcggga agccggatcg cgctatctga gccagggtgt catcgacgag  
 360  
 cgtggcgaga tcgttgccaa tcggcgcaag ctgaagccca cacacgttga gcgtacgac  
 420  
 tacggcgaag gcaacggaac cgatttctc acgcacgact tcgcgttcgg acgcgtcgg  
 480  
 ggattgaact gctgggaaca ttccaaccg ctacgaagt tcatgatgta cagcctcgg  
 540  
 gacgaggtcc acgttgcatc gtggcggcg atgtccctc ttcagccgga tgttttccaa  
 600  
 ctgagcatcg aagccaacgc gacggtcacc cgctcgtacg caatcgaagg caaaccttt  
 660  
 gtgctttgct cgacgcaggt gatcgacct agcgcgatcg aaactgtctg ctcaacgac  
 720  
 gaacagcgcg cactgttgcc gcaaggatgt ggctgggcg gcatttacgg ccggatgga  
 780  
 agcgagcttg cgaagcctct ggcggaagat gctgagggga tctgtacgc agagatcgat  
 840  
 ctggagcaga ttctgctggc gaaggtgga gccgatccgg tcgggcaact ttcgggcct  
 900  
 gacgtgctgt cgtccagtt cgaccgcgc aatcatacgc cagttcatcg catcgccatt  
 960  
 gacggtcgct tggatgtgaa taccgcagc gcgctggaga atttcgact gcgacaagcg  
 1020  
 gctgagcagg agcgtcaggc atccaagcgg ctcggaacga aactcttga acaatccct  
 1080  
 ctggctgaag aaccggtccc agcaaagtag  
 1110

<210> 5

<211> 369  
 <212> PRT  
 <213> Acidovorax facilis

<400> 5  
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 1 5 10 15  
 Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile  
 20 25 30  
 Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu  
 35 40 45  
 Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys  
 50 55 60  
 Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu  
 65 70 75 80  
 Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys  
 85 90 95  
 Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr  
 100 105 110  
 Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg  
 115 120 125  
 Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly  
 130 135 140  
 Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly  
 145 150 155 160  
 Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met  
 165 170 175  
 Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser  
 180 185 190  
 Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr  
 195 200 205  
 Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser  
 210 215 220  
 Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp  
 225 230 235 240  
 Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr  
 245 250 255  
 Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu  
 260 265 270  
 Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys  
 275 280 285  
 Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser  
 290 295 300  
 Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile  
 305 310 315 320

Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg  
325 330 335

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly  
340 345 350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala  
355 360 365

Lys

<210> 6  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Primer

<400> 6  
gacgcatatg gtttcgtata acagcaa  
27

<210> 7  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer

<400> 7  
cgacggatcc ttatggctac ttgctgg  
28

<210> 8  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Primer

<400> 8  
cggatccatg gtttcgtata acagcaagtt c  
31

<210> 9  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Primer

<400> 9  
ttatggctac ttgctggga ccg  
23

<210> 10  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Primer

<400> 10  
 tacatatggt ttcgtataac agcaagttc  
 29

<210> 11  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Primer

<400> 11  
 catctcgaga tggtttcgta taacag  
 26

<210> 12  
 <211> 23  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Primer

<400> 12  
 cactcgagct actttgctg gac  
 23

<210> 13  
 <211> 1110  
 <212> DNA  
 <213> *Acidovorax facilis*

<400> 13  
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 gacgcagacg caacgatoga caagtcgacg ggcatcatcg aagaagctgc ccaaaagggc  
 120  
 gcgagctcga tcgctttccc ggaagtatgc attccgggct acccctattg ggcgtggctc  
 180  
 gccgacgcta agtacagcct aagctttact tcacgctatc acgagaattc gttggagcta  
 240  
 ggtgacgacc gtatgcgctg cctccagctg gccgcgcgcg gcaacaaaaa cgcactcgtc  
 300  
 atgggctatt cggagcggga agccggatcg cgctatctga gccaggtggt catcgacgag  
 360  
 cgtggcgaga tcgttgccaa tcggcgcaag ctgaagccca cacacgttga gcgtacgac  
 420  
 tacggcgaag gcaacggaac cgatttcctc acgcacgact tcgcggttcgg acgcgtcggg  
 480

ggattgaact gctgggaaca tttccaacgc ctcagcaagt tcatgatgta cagcctcggt  
 540  
 gagcagggtcc acgttgcatc gtggccggcg atgtcccctc ttcagccgga tjttttccaa  
 600  
 ctgagcatcg aagccaacgc gacggtcacc cgctcgtacg caatcgaagg ccaaaccttt  
 660  
 gtgctttgct cgacgcaggt gatcgacatc agcgcgatcg aaacgttctg cctcaacgac  
 720  
 gaacagcgcg cactgttgcc gcaaggatgt ggctggggcg gcatttacgg ccgggatgga  
 780  
 agcgagcttg cgaagcctct ggcggaagat gctgagggga tcttgtagcg azagatcgat  
 840  
 ctggagcaga tctgctggc gaaggctgga gccgatccgg tcgggcacta ttcggcgctt  
 900  
 gacgtgctgt cggtcagtt cgaccgcgc aatcatcgc cagttcatcg catcggcatt  
 960  
 gacggtcgct tggatgtgaa tacccgcagt cgcgtggaga atttcgcact ggcacaagcg  
 1020  
 gctgagcagg agcgtcaggc atccaagcgg ctcggaacga aactctttga acaatccctt  
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 ctggctgaag aaccggtccc agcaaagtag  
 1110

<210> 14  
 <211> 369  
 <212> PRT  
 <213> Acidovorax facilis

<400> 14  
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 Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile  
 20 25 30  
 Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu  
 35 40 45  
 Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys  
 50 55 60  
 Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu  
 65 70 75 80  
 Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys  
 85 90 95  
 Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr  
 100 105 110  
 Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg  
 115 120 125  
 Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly  
 130 135 140  
 Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly  
 145 150 155 160  
 Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met  
 165 170 175  
 Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser

180 185 190

Pro Leu Gln Pro Asp Val Phe Gln Leu Ser Ile Glu Ala Asn Ala Thr  
195 200 205

Val Thr Arg Ser Tyr Ala Ile Glu Gly Gln Thr Phe Val Leu Cys Ser  
210 215 220

Thr Gln Val Ile Gly Pro Ser Ala Ile Glu Thr Phe Cys Leu Asn Asp  
225 230 235 240

Glu Gln Arg Ala Leu Leu Pro Gln Gly Cys Gly Trp Ala Arg Ile Tyr  
245 250 255

Gly Pro Asp Gly Ser Glu Leu Ala Lys Pro Leu Ala Glu Asp Ala Glu  
260 265 270

Gly Ile Leu Tyr Ala Glu Ile Asp Leu Glu Gln Ile Leu Leu Ala Lys  
275 280 285

Ala Gly Ala Asp Pro Val Gly His Tyr Ser Arg Pro Asp Val Leu Ser  
290 295 300

Val Gln Phe Asp Pro Arg Asn His Thr Pro Val His Arg Ile Gly Ile  
305 310 315 320

Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg  
325 330 335

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly  
340 345 350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala  
355 360 365

Lys

<210> 15  
<211> 1776  
<212> DNA  
<213> Acidovorax delafieldii

<400> 15  
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120  
ctatgacgtc tactccatac ttctgcaaga aaaagggggg gaaatttttc attccccaat  
180  
tattaggagg atcgtgtctaa tagtaaaggg caaacctcta ttttttatta ggtatagatg  
240  
tctaataatt aaatcagctc ggcgaatgcg tagcgtcgcg gcaaccagca caaggcaatt  
300  
ctgacagtga caccctctt aggagacgac cgtgggttcg tataacagca agttcctcgc  
360  
ggcaaccgtt caggcagagc cggatggct cgacgcagac gcaacgatcg acaagtcgat  
420  
cggcatcgc gaagaagctg cccaaaaggg cgcgagtcgt atcgctttcc cggaagtatt  
480  
cattccgggc taccctatt gggcgtggct cggcgacgtg aagtacagcc taagctttac  
540

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ttcacgctat caccagaatt cgttgagct aggtgacgac cgtatgcgtc g:ctccagct
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ggccgcgcg cgaacaaaaa tcgcaactcgt catgggctat tcggagcggg aagccggatc
660
gcgctatctg agccagggtg tcatcgacga gcgtggcgag atcgttgcca a:cggcgcaa
720
gctgaagccc acacacgttg agcgtaacgt ctacggcgaa ggcaacggaa c:gatttcct
780
cacgcacgac ttccgcttcg gacgcgtcgg tggattgaac tgctgggaa a:ttccaacc
840
gctcagcaag ttcatgatgt acagcctcgg tgagcaggtc cacgttgcat c:tgggccggc
900
gatgtccctt ettcagcgg atgttttcca actgagcatc gaagccaacg c:jacgggtcac
960
ccgctcgtac gcaatcgaag gccaaacctt tgtgctttgc tcgacgcagg t:atcggacc
1020
tagcgcgac gaaacgttct gccctaacga cgaacagcgc gactgttgc c:caaggatg
1080
tggctgggag cgcatttacg gcccgatgg aagcgagctt gcgaagcctc tggcggaaga
1140
tgctgagggg atctgttacg cagagatcga tctggagcag attctgctgg c:aaaggctgg
1200
agccgatccg gtcgggcact attcgcggcc tgacgtgctg tcggtccagt t:gacccgag
1260
caatcatacg ccagttcatc gcatcggcat tgacggtcgc ttggatgtga a:accgcgag
1320
tcgctgggag aatttcgac tgcgacaagc ggctgagcag gagcgtcagg catccaagc
1380
gctcgggaag aaactctttg aacaatcctt tctggctgaa gaaccgggtc cagcaagta
1440
gccataagtt gagagtcgag agatagtatc ggggaaagcc atctctggtc t:ccccctta
1500
ttctccaagc cgacatcacc gctgaaagcg ggtttctttg ctaccccgag t:tcgatccc
1560
gcacgcgctg cgcgtgagat ttgcgtcaga gcggacattc aagttgtgtg g:aaaggctgt
1620
ccagactgtc cagggaaaaa tccagttctt cactcgggtc aaggctcagc g:ttgtgctgc
1680
ggccgtgttc ctgtggccgc ctgacgaatg ccgtcctcag gccacaacgt c:jagcggtg
1740
ccaagtcacg gttgtgcgcc gccaccatgc agatct
1776

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<210> 16

<211> 1110

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A synthetic version of the nitrilase gene

<400> 16

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120
gcctctttga tcgctttccc agaagttttt attccagggt acccatactg g:cgctgggtg
180
ggtgacgtta agtactcttt gtcctttact tccagatata acgagaactc ttggagttg
240
ggtgacgaca gaatcgtag actgcaattg gctgcccgta gaacaaaaa t:gttttggtc
300

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atgggttatt cggagagaga agctggatct cgttacttgt cccaagtctt catcgacgag  
 360  
 agaggtgaga ttgttgcaaa tcgtcgtaag ttgaagccaa ctacagtga gcgtagcatc  
 420  
 tacggagaag gtaacggaac cgatttcttg actcacgact tcgccttcgg aagagttggg  
 480  
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 540  
 gagcaagtcc acgttgcttc ttggccagct atgtcccttc ttcagccaga tgttttccaa  
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 ttgtccatcg aagccaacgc caccgtcacc agatctctac ccacgaagg tcaaaccttt  
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 720  
 gaacagagag ctttgttgcc acaaggatgt ggttgggcaa gaatttcagg tccagatgga  
 780  
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 840  
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 900  
 gacgtcttgt ccgtccaggt cgaccctaga aaccacactc cagttcacag aattggattt  
 960  
 gacggtgatg tggatgttaa caccgatccc agagtcgaga acttcagact gagacaagct  
 1020  
 gctgagcagg agagacagcg ttctaagaga cttggaacta aacttttcga acaatctctt  
 1080  
 ttggctgaag aacctgtccc agccaagtaa  
 1110

<210> 17  
 <211> 84  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 oligonucleotide

<400> 17  
 catgaattca tggtttctta caactccaag ttcttggctg ctactgttca agctgagcca  
 60  
 gtttgggttg acgcagacgc tact  
 84

<210> 18  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 oligonucleotide

<400> 18  
 ttgatcgct ttcccagaag ttttcattcc aggttaccca tactgggcct ggttgggtga  
 60  
 cgtaaagtac tctttgtcct ttacttccag  
 90

<210> 19  
 <211> 90

<212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:Synthetic  
           oligonucleotide  
  
 <400> 19  
 aattggctgc ccgtagaaac aaaattgctt tggtcatggg ttattccgag ajagaagctg  
 60  
 gatctcgtaa cttgtcccaa gtcttcatcg  
 90  
  
 <210> 20  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:Synthetic  
           Oligonucleotide  
  
 <400> 20  
 gttgagcgta ccatctacgg agaaggtaac ggaaccgatt tcttgactca cgacttcgac  
 60  
 ttcggaagag ttggtggatt gaactgttgg  
 90  
  
 <210> 21  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 21  
 agtccacggt gcttcttggc cagctatgtc ccctcttcag ccagatgttt tccaattgtc  
 60  
 catcgaagcc aacgccaccg tcaccagatc  
 90  
  
 <210> 22  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 22  
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 60  
 gatgtggttg ggcaagaatt tacggtccag  
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 <210> 23  
 <211> 90

<212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 23  
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 60  
 cactactcca gacctgacgt cttgtccgtc  
 90  
  
  
 <210> 24  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 24  
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 60  
 gcaggagaga caggcttcta agagacttgg  
 90  
  
  
 <210> 25  
 <211> 84  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 25  
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 60  
 agttccaagt ctcttagaag cctg  
 84  
  
  
 <210> 26  
 <211> 90  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
           oligonucleotide  
  
 <400> 26  
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 60  
 ggtcgaactg gacggacaag acgtcaggtc  
 90  
  
  
 <210> 27  
 <211> 90

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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      oligonucleotide

<400> 27
tccaaatcga tctcagcgta caaaataccc tcagcatctt cagccaaagg cttggcaagg
60
tcagatccat ctggaccgta aattcttgcc
90

<210> 28
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      oligonucleotide

<400> 28
ggtttcgata gcagaaggtc caatgacctg ggtagagcaa aggacaaaag tttgaccttc
60
gatggcgtag gatctggtga cgggtggcgtt
90

<210> 29
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      oligonucleotide

<400> 29
gccaaagaagc aacgtggact tgctcaccca aggagtacat catgaaactta gacagagggt
60
ggaaatgttc ccaacagttc aatccaccaa
90

<210> 30
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      oligonucleotide

<400> 30
ccgtagatgg tacgctcaac gtgagttggc ttcaacttac gacgatttgc aacaattctca
60
cctctctcgt cgatgaagac ttgggacaag
90

<210> 31
<211> 90

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<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 31  
gtttctacgg gcagccaatt gcagtctacg cattctgtcg tcacccaact ccaaagagtt  
60  
ctcgtgatat ctggaagtaa aggacaaaga  
90

<210> 32  
<211> 90  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

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